#10/A
P78
9/24/02

MAILING CERTIFICATE

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington D.C. 20231 on

05 Sept 2002 by Barry L. Davison
Date Signature Printed name

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Markl et al.

Serial No.: 09/699,243

Filed: 27 October 2000

For: METHYLATION ALTERED DNA SEQUENCES AS MARKERS ASSOCIATED WITH HUMAN CANCER

Examiner: Jeanine A. Goldberg

Art Unit: 1634

Docket No.: 47675-14

Date: 05 September 2002

Box Non Fee Amendment
Assistant Commissioner for Patents
Washington, DC 20231

AMENDMENT A UNDER 37 C.F.R. § 1.111

Sir or Madam:

This Amendment A is in response to an Office Action dated 05 March 2002 for the above-identified patent application. Kindly extend the time for response three months, up to and including, 05 September 2002. A Request for a Three-Month Extension of Time and the required fee are enclosed. Kindly amend the above-identified patent application as follows:

IN THE CLAIMS:

Kindly cancel claims 5 and 6 without prejudice.

Applicants, pursuant to 37 C.F.R. § 121(c)(3), submit a "clean set" of all pending claims and requests cancellation of all previous versions of the re-presented claims:

('Clean set' of proposed claims:)

A1

1. (Amended) A diagnostic or prognostic assay for cancer, comprising:

(a) obtaining a tissue sample from a test tissue;

(b) performing a methylation assay on DNA from the tissue sample, wherein the methylation assay determines the hypermethylation state of a CpG dinucleotide within at least one DNA sequence selected from the group consisting of sequences of SEQ ID NOS:34-38, sequences having a nucleotide sequence at least 98% identical to sequences of SEQ ID NOS:34-38, CpG island sequences associated with sequences of SEQ ID NOS:35-38, and CpG island sequences associated with sequences having a nucleotide sequence at least 98% identical to sequences of SEQ ID NOS:35-38, wherein a CpG island sequence is associated with the sequence of a particular SEQ ID NO if the CpG island sequence is a sequence of genomic DNA that is contiguous in the genome with, and encompasses at least one nucleotide of the particular SEQ ID NO sequence, and wherein the particular SEQ ID NO sequence is itself a portion of a larger CpG island sequence that satisfies the criteria of having both a frequency of CpG dinucleotides corresponding to an Observed/Expected Ratio >0.6, and a GC Content >0.5; and

(c) determining a diagnosis or prognosis based, at least in part, upon the methylation state of the CpG dinucleotide within the DNA sequence, wherein the determined methylation state is either hypermethylation or normal methylation, and wherein the cancer is bladder or prostate cancer.

2. (Amended) The diagnostic or prognostic assay of claim 1 wherein the DNA sequence is a sequence selected from the group consisting of CpG island sequences associated with sequences of SEQ ID NOS:35-38, and CpG island sequences associated with sequences having a nucleotide sequence at least 98% identical to sequences of SEQ ID NOS:35-38.

3. (Amended) The diagnostic or prognostic assay of claim 2 wherein the DNA sequence is a sequence selected from the group consisting of CpG island sequences associated with sequences of SEQ ID NOS:35-38.

4. (Amended) The diagnostic or prognostic assay of claim 1 wherein the methylation assay procedure is selected from the group consisting of MethyLight, MS-SNuPE, MSP, MCA, COBRA, and combinations thereof.

A2

7. (Amended) A kit useful for the detection of a methylated CpG-containing nucleic

AZ cancelled

acid comprising a carrier means containing one or more containers comprising:

(a) a container containing a probe or primer which hybridizes to any region of at least 12 nucleotides of a sequence selected from the group consisting of SEQ ID NOS:34-38, and sequences having a nucleotide sequence at least 98% identical to sequences of SEQ ID NOS:34-38; and

(b) additional standard methylation assay reagents required to affect detection of methylated CpG-containing nucleic acid based, at least in part, on the probe or primer.

8. (Amended) The kit of claim 7, wherein the additional standard methylation assay reagents are standard reagents for performing a methylation assay from the group consisting of MethyLight, MS-SNuPE, MSP, MCA, COBRA, and combinations thereof.

9. (Amended) The kit of claim 7, wherein the probe or primer comprises at least 12 nucleotides of a sequence selected from the group consisting of SEQ ID NOS:34-38, and sequences having a nucleotide sequence at least 98% identical to sequences of SEQ ID NOS:34-38.

10. (Amended) An isolated nucleic acid molecule comprising a methylated or unmethylated polynucleotide sequence selected from the group consisting of sequences of SEQ ID NO:34, SEQ ID NO:37, SEQ ID NO:38, and sequences having at least 98% sequence identity thereto.

11. The nucleic acid of claim 10, wherein the nucleic acid is methylated.

12. The nucleic acid of claim 10, wherein the nucleic acid is unmethylated.

REMARKS

Claims 1-12 are pending.

Applicants have herein cancelled *dependent* claim 5 and 6 without prejudice, and limitations thereof have been recited in *independent* claim 1 (Amended).

The claims have been amended to reflect the restriction election of SEQ ID NOS:34-38. No new matter has been added.

Applicants acknowledge the Examiner's current rejections under 35 U.S.C. § 112 *first* and *second* paragraphs, and under 35 U.S.C. § 102. Applicants have responsively amended the *independent* and *dependent* claims, such that the scope of the currently amended claims is commensurate with the written description and enablement provided by the originally filed specification, and so that the claims are free of the art.

Applicants respectfully request reconsideration of the above-identified patent application in view of the foregoing amendments and following remarks. Applicants respectfully request entry of the present Amendment A, and allowance of all pending claims 1 (Amended), 2 (Amended), 3 (Amended), 4 (Amended), 7 (Amended), 8 (Amended), 9 (Amended), 10 (Amended), 11 and 12. No new matter has been added.

Formalities/Inventorship

The inventorship status of the pending claims remains unchanged, despite the restriction/election of SEQ ID NOs:34-38. Applicants thank the Examiner for the inquiry/reminder regarding the duty to update inventorship status in view of the restriction/election.

Rejections under 35 U.S.C. § 112, ¶1

Written Description:

The Examiner rejected claims 1-12 under 35 U.S.C. § 112, first paragraph, "as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention."

Specifically, the Examiner, citing *The Regents of the University of California v. Eli Lilly* 43 USPQ2d 1398-1412 (Fed Cir. 1997), alleges that while the claims recite a genus of "DNA

sequences having at least 90% identity with SEQ ID NO:34-38, CpG island sequences associated with SEQ ID NO:34-38, CpG island sequences associated with sequences having a nucleotide sequence at least 90% identical to SEQ ID NO:34-38, and combination s thereof," "applicants have not adequately disclosed the relevant identifying characteristics of a representative number of species within the claimed genus (*see* Office Action of 05 March 2002 at pages 2-4).

In reaching this conclusion, the Examiner appears to be following a cDNA/EST-type analysis of applicant's disclosed sequences, as evidenced by the reference to Example 7 of the Written Description guidelines (relating to ESTs), and emphasizing that applicants have not disclosed "any [complete] genomic DNA sequences and particularly have not adequately disclosed any intron or regulatory sequences." Moreover, the Examiner asserts that "the specification does not appear to have described any sequences which are 90% identical with SEQ ID NO:34-38 and have the same asserted function for detecting cancer, and does not appear to define what "associated" means (*Id*)."

Applicants respectfully traverse the Examiner's assertion that applicants have not adequately disclosed the relevant identifying characteristics of a representative number of species within the claimed genus that are less than 100% identical to applicants SEQ ID NOS:34-38.

First, applicants call the Examiner's attention to the fact that *independent* claim 1, as originally filed, already recites "consisting of sequences," rather than open-ended comprising language that is precluded in the context of Example 7 of the Written Description guidelines.

Second, the relevant identifying characteristics of applicant's "associated" CpG-island sequences are clearly defined in the specification (*see* Specification; definition of CpG island at page 4, line 36 to page 5, line 5). Moreover, inclusion of such associated CpG island sequences within the claim scope is entirely supported by the specification, which states the art-recognized principal that "the methylation state of a portion of a given CpG island is generally representative of the island as a whole...." (*see* Specification at page 8, lines 22). That this conceptual "association" was indeed within the possession of the inventors at the time of filing is supported by the teachings of applicant's own research article published prior to the filing of the present application (*see* Liang et al., *Cancer Research* 60:4907-4912 (01 September 2000), at the first column of page 4911; attached hereto as APPENDIX B).

Third, applicants have in fact described sequences *having diagnostic utility* that are less than 100% identical to applicants SEQ ID NOS:34-38. Collectively, SEQ ID NOS:34-38 in

applicant's originally filed Sequence Listing include 22 variable nucleotide positions (listed as "n") out of 1,248 listed nucleotide positions (about 2%). These "n" positions are thus effectively disclosed as A, G, C, or T, and represent written description for a sequence *genus* having about 2% variability in terms of identity, and having the disclosed utility.

Applicants, in response to the Examiner's comments, have nonetheless amended *independent* claim 1 to reflect the nature of the above described "associated" sequences and the extent of variability by reciting "sequences having a nucleotide sequence at least 98% identical to sequences of SEQ ID NOS:34-38, CpG island sequences associated with sequences of SEQ ID NOS:35-38, and CpG island sequences associated with sequences having a nucleotide sequence at least 98% identical to sequences of SEQ ID NOS:35-38" (*see* APPENDIX A, attached hereto for marked-up claims).

Moreover, claim 1 has also been amended to clarify and further define the nature of the "associated" sequence and to limit such associations to those SEQ ID NO sequences that are themselves portions of CpG islands by reciting "wherein a CpG island sequence is associated with the sequence of a particular SEQ ID NO if the CpG island sequence is a sequence of genomic DNA that is contiguous in the genome with, and encompasses at least one nucleotide of the particular SEQ ID NO sequence, and wherein the particular SEQ ID NO sequence is itself a portion of a larger CpG island sequence that satisfies the criteria of having both a frequency of CpG dinucleotides corresponding to an Observed/Expected Ratio >0.6, and a GC Content >0.5." (*Id*).

Conforming amendments have been made to claims 2, 3, 7, 9 and 10.

Applicants amendments have thus narrowed the scope of the claimed genus commensurate with the written description by increasing the percentage of required sequence identity to 98%, by limiting the associated CpG-island sequences to those associated with SEQ ID NOS that are themselves CpG islands (*i.e.*, SEQ ID NOS:35-38, but not SEQ ID NO:34), and by clarifying the nature of the "associated" sequences.

Applicants, therefore, respectfully request withdrawal of the Examiner's 35 U.S.C. § 112, first paragraph (written description) rejection with respect to claims 1 (Amended), 2 (Amended), 3 (Amended), 4 (Amended), 7 (Amended), 8 (Amended), 9 (Amended), 10 (Amended), 11 and 12.

Enablement:

The Examiner rejected claims 1-12 under 35 U.S.C. § 112, first paragraph, "as containing subject matter which was not described in the specification in such a way as to *enable* one skilled in the relevant art to make or use the invention (*see* Office Action of 05 March 2002, at page 5).

Specifically, and ***first***, the Examiner asserts that applicant's own statements in the specification undermine and contradict the claimed utility (*see* Office Action of 05 March 2002, at pages 5-6, citing, *inter alia*, page 2 lines 31-35).

Specifically and ***second***, the Examiner asserts that the specification teaches that SEQ ID NOS:34-38 are hypermethylated as opposed to hypomethylated, whereas the claims recite broader language that includes hypomethylation (*Id*, at page 7).

Specifically, and ***third***, the Examiner asserts that the disclosed methylation analysis relating to SEQ ID NOS:34-38 was performed *only* in the context of prostate and bladder cancer tissue, and therefore does not enable diagnosis of any and all cancers, based on the same methylation differences (*Id*, at pages 7 and 8).

Specifically, and ***fourth***, the Examiner asserts that the specification has not taught a predictable correlation between nucleic acids which are ***associated*** with SEQ ID NOS:34-38' (*Id*, at page 8).

Applicants respectfully traverse the Examiners above-described rejections with respect to the ***first*** and ***fourth*** assertions, based on the teachings of applicant's originally filed specification, and the knowledge and skill in the relevant art at the time of filing. However, applicants have responsively amended the claims in view of the Examiner's comments under the ***second*** and ***third*** assertions.

First, with respect to the Examiners *first* assertion, applicants respectfully contend that the Examiner has inappropriately misconstrued applicant's quoted statements. Significantly, applicant's statement at issue on page 2 of the specification says that the mere knowledge that any (any unidentified sequence or gene) altered methylation states exist (or that methylation can affect particular gene expression, in the abstract sense) does not enable detection, and rather this altered methylation state (or altered expression) must be correlated to ***specific*** sequences or genes, and in turn correlated with cancer. This statement was part of applicant's "Background" section, and the intended meaning is clearly to indicate that methylation analyses and correlations need to be made with ***specific*** sequences or genes.

In fulfillment of this need, applicant's specification teaches and discloses altered methylation states of *particular* sequences and correlates these *particular* markers with cancer, and in particular with bladder and prostate cancer. Applicants are not required to show that such altered methylation is *causative* of cancer, but rather show, as has in fact been determined, that a distinguishing (with respect to normal tissue) association or correlation exists between the methylation state of particular CpG dinucleotide sequences and cancer.

Second, with respect to the Examiners *second* assertion, applicants have amended step (c) of *independent* claim 1 to recite "determining a diagnosis or prognosis based, at least in part, upon the methylation state of the CpG dinucleotide within the DNA sequence, wherein the determined methylation state is either hypermethylation or normal methylation" (*see* Appendix A for marked-up claims). No new matter has been added.

Third, with respect to the Examiners *third* assertion, applicants have cancelled *dependent* claim 6, and further amended step (c) of *independent* claim 1 to recite "and wherein the cancer is bladder or prostate cancer" (effectively incorporating the bladder and prostate limitations of the Markush group of claim 6 into claim 1) (*see* Appendix A for marked-up claims). No new matter has been added.

Fourth, with respect to the Examiners *fourth* assertion, applicants have, as described in detail above in rebuttal to the Examiner's Written Description based rejection, clarified the nature of applicant's recited "associated" CpG island sequences, and has cited support in the specification and prior art (including applicant's own literature publication).

Specifically, inclusion of such "associated" CpG island sequences within the claim scope is entirely supported by the specification, which states the art-recognized principal that "the methylation state of a portion of a given CpG island is generally representative of the island as a whole...." (*see* Specification at page 8, lines 22). Moreover, that this conceptual "association" was indeed within the possession of the inventors at the time of filing is supported by the teachings of applicant's own research article published prior to the filing of the present application (*see* Liang et al., *Cancer Research* 60:4907-4912 (01 September 2000), at the first column of page 4911; attached hereto as APPENDIX B).

Applicants, therefore, respectfully request withdrawal of the Examiner's 35 U.S.C. § 112, first paragraph (enablement) rejection with respect to claims 1 (Amended), 2 (Amended), 3 (Amended), 4 (Amended), 7 (Amended), 8 (Amended), 9 (Amended), 10 (Amended), 11 and 12.

Rejections under 35 U.S.C. § 112, ¶2

Claims 1-6, and 8-9 were rejected by the Examiner under 35 U.S.C. § 112 second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention (*see* OA of 05 March 2002, at page 9, para 4.).

Specifically, the Examiner alleges **(a)** that claims 1-6 are indefinite, over the recitation "derived from" (*Id*). Specifically, the Examiner suggests that applicants delete the word "derived."

The Examiner further alleges **(b)** that claims 1-6 are indefinite in the recitation of "associated" in relation to CpG island sequences (*Id*).

Additionally, the Examiner alleges **(c)** that claims 1-6 are indefinite in the recitation of "combinations thereof," which does not distinguish combinations of separate distinct sequences from "scrambled" sequence combinations."

The Examiner further alleges **(d)** that claim 8 is indefinite in the recitation of "MSP MCA," and likely need a coma between "MSP" and "MCA."

Finally, the Examiner further alleges **(e)** that claim 9 is indefinite over the phrase "at least about," which has been held to be indefinite.

(a): With respect to (a) above, the applicants have clarified *independent* claim 1 by herein deleting the word "derived," as suggested by the Examiner. Applicants thank the Examiner for this suggestion. No new matter has been added (*see* Appendix A, attached hereto, for 'marked-up' claims).

(b): With respect to (b) above, applicants have clarified the meaning of "associated" by herein amending *independent* claim 1 to recite "wherein a CpG island sequence is associated with the sequence of a particular SEQ ID NO if the CpG island sequence is a sequence of genomic DNA that is contiguous in the genome with, and encompasses at least one nucleotide of the particular SEQ ID NO sequence, wherein the particular SEQ ID NO sequence is itself a portion of a larger CpG island sequence that satisfies the criteria of having both a frequency of CpG dinucleotides corresponding to an Observed/Expected Ratio >0.6, and a GC Content >0.5." Support for this amendment is found throughout the Specification, and in particular at page 8,

lines 18-22. No new matter has been added (*see* Appendix A, attached hereto, for 'marked-up' claims).

(c): With respect to (c) above, applicants have removed the ambiguity associated with recitation of "and combinations thereof" by deleting this phrase from *independent* claim 1, and reciting in its place "within at least one DNA sequence selected from the group consisting of...," which more clearly describes the intended meaning of combinations thereof. No new matter has been added (*see* Appendix A, attached hereto, for 'marked-up' claims).

(d): With respect to (d) above, applicants have, in *dependent* claim 8, inserted a coma between "MSP" and "MCA," as suggested by the Examiner, and as supported in the Specification at page 5, under "Definitions." Although not specifically requested by the Examiner, a similar conforming amendment was made to *dependent* claim 4. No new matter has been added (*see* Appendix A, attached hereto, for 'marked-up' claims).

(e): With respect to (e) above, applicants have substituted the phrase "at least 12 nucleotides" in place of "at least about 12 to 15 nucleotides" in *dependent* claim 9.

In summary, with respect to **(a)**, **(b)**, **(c)**, **(d)**, and **(e)** above, applicant respectfully requests withdrawal of the Examiner's 35 U.S.C. § 112 *second* paragraph indefiniteness rejections with respect to claims 1 (Amended), 2 (Amended), 3 (Amended), 4 (Amended), 8 (Amended) and 9 (Amended). No new matter has been added.

Rejections under 35 U.S.C. § 102

Claims 7-9 were rejected by the Examiner under 35 U.S.C. § 102 as being anticipated by Herman et al. (US Pat. 5,786,146, July 28, 1998) (*see* Office Action of 05 March 2002, at page 11).

Specifically, the Examiner asserts that the primer corresponding to SEQ ID NO:46 of Herman et al. would hybridize to nucleotides 212-219 (8 nucleotides) of applicant's SEQ ID NO:34 sequence, and thus comprise at least about 12 nucleotides which are at least about 90% identical to applicant's SEQ ID NO:34. Alternatively, the Examiner asserts that the Herman et al. primer comprises 10 nucleotides which are 90% identical to SEQ ID NO:34 (*Id*).

Applicants have, in response to the Examiner's comments, amended independent claim 7 to recite "containing a probe or primer which hybridizes to any region of at least 12 nucleotides of

a sequence selected from the group consisting of SEQ ID NOS:34-38, and sequences having a nucleotide sequence at least 98% [90%] identical to sequences of SEQ ID NOS:34-38."

Additionally, conforming amendments have been made to *dependent* claim 9, which now recites "at least 12 nucleotides" in place of "at least about 12 to 15 nucleotides."

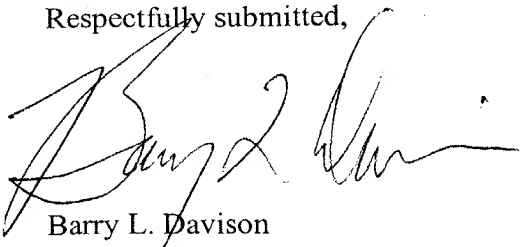
These amendments distinguish the present invention from that of Herman et al. by requiring hybridization to at least a 12 nucleotide region, or regions that are at least 98% identical to such a region. The amendments are supported, *inter alia*, by the originally filed claims, and in particular claim 9. No new matter has been added.

Applicants, therefore, respectfully request withdrawal of the Examiner's 35 U.S.C. § 102 anticipation rejection under Herman et al. with respect to claims 7 (Amended), 8 (Amended) and 9 (Amended).

Conclusion

In view of the foregoing amendments and remarks, applicants respectfully request reconsideration of the claimed invention and allowance of all pending claims 1 (Amended), 2 (Amended), 3 (Amended), 4 (Amended), 7 (Amended), 8 (Amended), 9 (Amended), 10 (Amended), 11 and 12.

Respectfully submitted,



Barry L. Davison
Attorney for Applicants
Registration No. 47,309

Davis Wright Tremaine
2600 Century Square
1501 Fourth Avenue
Seattle, Washington 98101-1688
Telephone (206) 628 7621
Facsimile (206) 628 7699